Response to IRSP. Project ID 35027: Evaluation of Two Captive Rearing Methods for Assisting with Recovery of Naturally Spawning Populations of Steelhead and Coho Salmon.

August 23, 2002

TO: ISRP

FR: Don Campton (Ph.D., Geneticist), Principal Investigator
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RE: Preliminary review comments of ISRP, ISRP 2002-13, August 2, 2002

We thank the ISRP for their detailed comments and constructive recommendations in their review of our proposal. We truly thank the ISRP for pointing out potential opportunities of our proposed work. We agree with most of their recommendations.

The biggest concern of the ISRP regarding our proposal was that we were potentially missing an opportunity to evaluate the effects of the culture environment on the biology and performance of the fish in our hatchery. We acknowledge this oversight in our proposal, primarily because we were most concerned with explicitly addressing RPAs 182, 183, and 184. However, some facility limitations would prevent us from completely adopting all of the ISRP's recommendations. Their specific recommendations are discussed below.

a) DNA sampling the original parr collected so that genetic variation in the source population is known.

Response: We agree. This recommendation of the ISRP is actually addressed in our proposal under *Task 1f: Estimate allele frequencies at 10-20 nuclear DNA microsatellite loci for NOR* [natural-origin] *steelhead from Abernathy Creek*. Under this task, we state we will collect fin clips from "a minimum of 100 fish of each brood year." However, as proposed, those fin clips would be collected strictly at the time of spawning, or at the adult stage.

We will continue to collect fin clips from all captively-reared adults that we spawn (age 3), but we will also collect fin clips from a random subsample of 100 age 0+ juveniles/parr approximately one month after capture from Abernathy Creek. This will allow us to assess the potential genetic effects of natural selection under the culture environment from the time of capture (age 0+ parr) to sexual maturation and adulthood (age 3). We will clip the distal portion of the anal from each of 100 parr, and place the fin tissue in a 2.0 ml microfuge tube (with screw cap) filled with 1.0 ml of 100% (200 proof) ethyl alcohol. We will store the fin clips at room temperature prior to DNA extraction. Procedures for DNA extraction and collection of allele frequencies at 10-20

nDNA microsatellite loci will follow the same general procedures cited by Paul Moran and Robin Waples (P.I's.) under Project No. 198909600.

The USFWS is currently working collaboratively with Paul Moran of NMFS on a genetic study of steelhead populations throughout the lower Snake River, with special emphasis on the Grande Ronde River. In addition, the USFWS has recently built a 900 square foot molecular genetics laboratory at the Abernathy Fish Technology Center. Our DNA procedures for steelhead will duplicate those developed by NMFS and currently used by Paul Moran in our collaborative steelhead studies in the Grande Ronde and Imnaha Rivers.

b) Maintaining the families in individual rearing tanks until they are large enough to tag (CWT and/or PIT tags), sample families before pooling.
c) PIT tag at least 100 individuals per family before pooling in the raceways, this will facilitate studying family responses to culture (variation of growth – task 1.d.—cannot be observed from observations of mean size of experimental groups.)

Response: This recommendation will be difficult to follow verbatim. Although the ISRP did not specify the "question" or specific "data" to be obtained via the above two recommendations, we assume their recommendations were motivated by a desire to measure phenotypic traits (see recommendation "f" below) within and between families to estimate potential between-family natural selection resulting from the culture environment.

Two factors will make it difficult for us to follow these recommendations completely. (1) We could accomplish these tasks for, at most, 50 families (instead of 100) based on our current facilities and demands of other projects. (2) However, our 4-foot circular tanks in our hatchery building are plumbed with well water (12^0 C), not surface (Abernathy Creek) water; consequently, post-hatch growth from first feeding to tag size (approx. 100 mm) will not mimic the growth patterns of fish in raceways which are plumbed with surface (Abernathy Creek) water. Major modifications and expenses would be necessary to set-up (or replumb) individual, 4-ft holding tanks with creek water.

In addition to recommendations "b" and "c" of the ISRP, a recently obtained result from our preliminary work to date has provided additional motivation for a slight change in study design. As proposed, we would spawn 100 captively-reared males and 100 captively-reared females to generate 100 full-sib families for release. We followed this protocol during the winter and spring of 2002 for captively-reared, age 3 adults (BY1999) that were collected as parr from Abernathy Creek during the early fall of 1999. Fecundity and fertilization success were excellent for most fish, with many captively-reared females producing more than 1,500 eggs (max. = 2,590). We culled each full-sib family to a maximum of 1,000 eyed eggs (or swim-up fry) to more equalize family size. Despite this culling, we ended up with over 70,000 age 0+ fry at the time of ponding. We recently culled this latter number down to approximately 25,000 fry with the goal of potentially releasing 20,000 age 1+ steelhead into Abernathy Creek. We appear to be able to produce large numbers of progeny steelhead for release from captively-reared juveniles.

Based on recommendations "b" and "c" from the ISRP and the apparently high fecundity and fertility of captively-reared fish to date, we are proposing the following modification to project design.

Task 2a: Spawn captively-reared, natural-origin adults. We will spawn 50 captively-reared males and 50 captively reared females to produce 50 full-sib families (instead of 100). Each full-sib family will be culled to a maximum of 600 eyed eggs for a maximum total of 30,000 eyed eggs.

Task 2b: Rear progeny of captively-reared parents to one year of age and/or the smolt stage. At the time of swim-up and ponding, a random subsample of 100 swim-up fry from each full-sib family will be transferred to separate 4-ft circular tanks (50 tanks total) in our hatchery building plumbed with well water. The remaining fry from each family will be transferred to an outdoor raceway supplied with Abernathy Creek water. Fish in the 4-ft. circular tanks will be raised to a minimum size of 100mm FL, tagged with 23 mm *extended range* PIT tags, and then transferred to the outdoor raceway for subsequent grow-out with their siblings.

d) Do not fin clip the fish as electronic sampling for blank wire will avoid the mortality associated with these fin clips.

Response: We agree. We will not clip the fins of any fish as a permanent mark for future identification. All fish will be given a blank wire tag so that released fish can be identified by scanning an electronic wand. This scanning will occur when fish are intercepted by screw trap during the smolt outmigration period (smolt trapping conducted by WDFW) and when upstream-migrating adults are trapped at the Abernathy Fish Technology Center.

e) Incorporate culture regimens (diet, ration, schedules, etc.) that achieve natural growth trajectories of parr and pre-smolts rather than regimens that "Maximize… growth rate and minimize the variance in growth rate" – task 1e of the proposal. Physiological fitness of smolts (by Dickhoff and others) suggests that the traditional growth-maximizing regimens may be inappropriate for supplementation programs.

Response: We agree. However, task 1e deals explicitly with the captive-rearing of the wild-caught, NOR parr that are raised to sexual maturity. Those fish will not be released. The goal there is to simply maximize survival and growth to produce the maximum number of sexually mature fish after three years of age (2.5 years of captive rearing).

The ISRP's comments are appropriate, though, for the fish we release. These latter fish are the progeny of the captively-reared adults. We do intend to follow a "natural growth" regimen for these latter fish . First, the vast majority of the fish will be on Abernathy Creek water from "swim-up" to release, and thus, will experience natural fluctuations in water temperature. This will substantially decrease food demands during the winter. Second, we will be using demand feeders primarily but supplementing demand feeding with belt feeding and hand feeding during the spring and summer months. We will suspend supplemental feeding in the late fall, and then resume supplemental feeding in the early spring when water temperatures and photoperiod begin increasing at accelerating rates. We have not had time to collate our own stream temperature data yet, but will do so when selecting cut-off and start-up dates for supplemental feeding. We will consult with Walt Dickhoff and his staff at NMFS for additional guidance and suggestions.

f) Sample phenotypic traits of the PIT tagged fish as they are being released from the raceways as smolts including physiological assessments such as those proposed for coho smolts (task 3.c).

Response: We agree. Steelhead are typically released between May 1 and May 15. Beginning approximately one month prior to release (April 1-7), we will sort through all age 1 fish representing the previous brood year. All fish will be scanned for PIT tags. The fork length of each fish will be measured to assess potential between-family selection for growth and size variation among families prior to release. In addition, we will measure fork lengths and obtain approximately a 0.5 cu. cm clip of tissue from the distal portion of the anal fin from 500-1,000 randomly selected fish that do NOT have PIT tags (exact sample size pending power analysis). These latter fish will be genotyped at 10-20 nDNA microsatellite loci, and their family I.D.s determined by comparing their multi-locus genotypes to those of the 50 male and 50 female parents that were spawned (Task 2a). We will thus have two measures of between-family survival and growth: (1) for PIT-tagged fish and (2) for fish that were not PIT-tagged. The cost of the DNA analysis is approximately $30-$50 per fish which places an upper limit on the total number of fish that can be genotyped to assess between family survival and yet achieve adequate power. PIT-tagged fish will subsequently be detected by the remote antennas described in Project No. 35060. A subsample of 5 PIT tagged fish from each family will be sacrificed to estimate gill $Na^+ K^+$ ATPase activity, seawater tolerance, plasma cortisol, prolactin, and thyroid hormone levels (see Task 3c of proposal for details).

g) Use the barrier fence to divert all adult steelhead through the facility and to electronically sample for CWT and PIT tagged fish.

Response: We agree. This is Objective 6 of our proposal and is an integral component of the overall proposal. We would then pass equal numbers of tagged (blank wire) and untagged (natural-origin) steelhead upstream of our weir to assess natural reproductive success of all adults passed upstream. All adults passed upstream will be given an opercle punch. We will measure lengths, and obtain fin clips and scale samples from all fish passed upstream. We will also collect additional fin clips and scales from tagged (blank wire) fish not passed upstream for a maximum of 1,000 tagged adults returning to our facility. Based on the DNA genotypes of these latter fish, we would assess between family survival and return rates for steelhead released from the Abernathy Fish Technology Center. We also plan to monitor the upstream migration of coho salmon to better understand the status of this latter species.

h) Incorporate truly randomized mate-assignment protocols (task 2a) (ISRP cited a previous study by Quinn et al. showing that mate assignments are not random when adults are selected for mating and spawning).

Response: We agree. The spawning of BY1999 steelhead during the winter and spring of 2002 was very protracted; it began in late January and continued through early May. Spawning typically occurred once a week. In response to the ISRP's concern, all gametes will be individually stripped into separate plastic, Ziploc bags. Bags of milt and eggs will then be combined randomly in the hatchery after all adults

have been sorted and mature gametes stripped into Ziploc bags. Eggs will be fertilized with no knowledge of the size or condition of the adult fish. This will achieve random mating.

i) Consider how to sample and/or use kelts that will be produced and how to manage the barrier fence when the kelts are moving downstream.

Response: We cannot address this issue at this time. We need further clarification from the ISRP regarding the purpose of sampling kelts. Potential repeat spawners will be intercepted on their return, upstream migration. Nevertheless, we will assemble a meeting of our FWS field personnel and local WDFW personnel to determine the logistic feasibility of sampling kelts if the ISRP can justify the purpose or goal of such sampling.

Regarding downstream passage of kelts, we will turn off our electric fence barrier at the end of the upstream migration period of steelhead (May-September). Downstream passage of kelts towards the end of the upstream migration period (mid-April thru May) can also be facilitated by turning the weir off after sunset and turning the weir back on just before sunrise via timers (or photocells). We will need to collect preliminary data on adult return timing in Abernathy to better identify these migration periods.

Final comments of ISRP

In its final comments, the ISRP noted that the coho portion of our proposal (hatchery overwintering) is, in their opinion, "low priority." We agree that it is a "low priority" compared to the "high" to "medium-high" priority of the other components (steelhead broodstock study, replacement of electronic fence in Abernathy Creek, development of a Tier 3 M&E site). Consequently, we will defer the coho overwintering portion of our proposal (Objective #3) to a future proposal or study. The cost savings of this portion of the proposal are compensated by the extra costs associated with the work recommended by the ISRP.

Once again, we thank the ISRP for their detailed and very constructive recommendations for our proposed work..